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Computerized Assessment of Human Neurotoxicity Sensitivity to Nitrous Oxide Exposure

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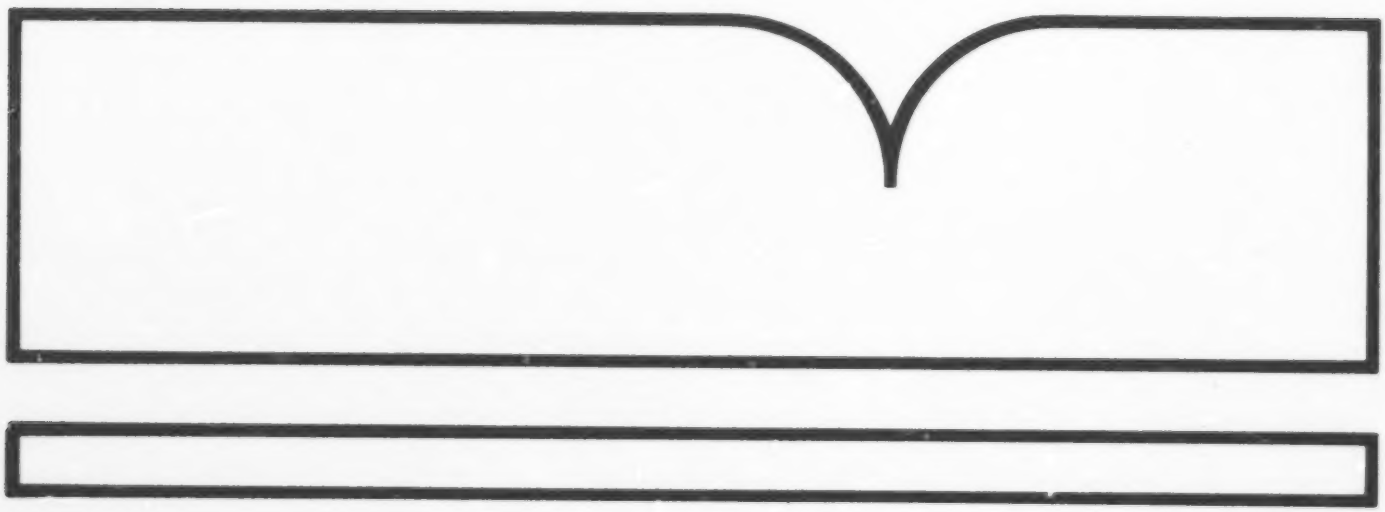

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16. ABSTRACT

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COMPUTERIZED ASSESSMENT OF HUMAN NEUROTOXICITY: SENSITIVITY TO NITROUS OXIDE EXPOSURE

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Computerized assessment of human neurotoxicity: Sensitivity to nitrous oxide exposure

We recently developed a flexible, portable, computer-based neurobehavioral evaluation system (NES) to standardize data collection in epidemiologic field studies of individuals at risk for neurobehavioral toxicity. The current study was performed to examine the system's sensitivity to subtle neurobehavioral impairment induced pharmacologically in normal subjects. Twelve men 18 to 36 years old were tested with the NES three times, in an initial training session followed by separate drug and control sessions in randomized order. During drug sessions subjects received a combination of 20% nitrous oxide and 80% oxygen through a nasal mask. In control sessions subjects received 100% oxygen. Nine NES tests evaluating psychomotor performance, visuospatial ability, memory, and mood were administered in approximately 40 minutes. Nitrous oxide exposure impaired performance on three tests: continuous performance, symbol-digit substitution, and finger tapping. Another test, pattern memory, showed a trend toward impairment, while the remainder were not affected by the drug. These data are consistent with previous reports that the threshold effect of nitrous oxide is a decrement in psychomotor speed. The findings indicate that our NES is sensitive to short-term impairment in central nervous system function of the type induced by a number of neuroactive agents. In view of its efficiency in data acquisition and analysis, computerized neurobehavioral testing appears to be a very promising method for evaluation of effects of a wide range of pharmacologic agents. (CLIN PHARMACOL THER 38:656-660, 1985.)

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Neurobehavioral tests have long been recognized as powerful tools in the assessment of functional impairment in the central nervous system (CNS) produced by a variety of causes, including naturally occurring disease, head trauma, or exposure to neurotoxic agents.[9] One recent development that promises to enhance the utility and expand the uses of neurobehavioral testing (for example, in fields such as neurobehavioral toxicology) has been tests that can be administered by computer. As compared with traditional methods, a computer-based neurobehavioral evaluation system (NES) offers the advantages of standardization of testing conditions, ease of data acquisition, reduced need for extensive training of testing personnel, reduced potential for experimenter bias, extremely precise response measurement, and high subject or patient acceptance.[3,7] We recently developed one such system, described in detail elsewhere,[2] for use in epidemiologic studies of populations occupationally exposed to neurotoxic agents. This system is intended to be capable of detecting subclinical, subtle CNS functional impairment. It is not designed to provide information on localization of functional impairment or specific diagnoses.

Before testing systems such as our NES can win wide acceptance as research instruments, a number of questions about their utility must be satisfactorily answered. The most important of these are issues of the reliability and validity of an evaluation system.[1] Such systems should provide measures that are reliably obtained, stable over time, and correlate well with data obtained from standard nonautomated techniques. These issues are the subject of ongoing research.[3] Of course, a test system must also detect functional impairment with a reasonably high degree of sensitivity. The purpose of

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our current study was to evaluate the sensitivity of the NES to a pharmacologic treatment producing subtle alteration in the functional status of the CNS. The treatment chosen was administration of a minimally suprathreshold dose of nitrous oxide, a safe, short-acting inhalation anesthetic with previously described CNS depressant properties.[6] Nitrous oxide is of particular interest as a test agent because of concern over possible occupational exposure to the drug among surgical and dental personnel,[10] and because its acute effects are analogous to those of CNS-depressant organic solvents used widely in industry.[11] Furthermore, demonstration of NES sensitivity to nitrous oxide would suggest potential utility of this system not only in epidemiologic field studies, but also in clinical research on neurobehavioral effects of a variety of drugs with varying modes of action.

METHODS

Subjects. Subjects were twelve men 18 to 36 years old ($\bar{X} \pm SD = 23 \pm 6.5$ years) who gave informed consent. The study was approved by the human subjects committee of the institution. Subjects were healthy and not taking any medication as determined by a medical history questionnaire. Subjects were undergraduate and graduate students and were paid for their participation.

Testing procedure. Each subject was tested on three separate occasions, beginning at the same time of day, over a 2-week period. Testing took place in an isolated room (82 ft^2) that was quiet and had moderately low fluorescent illumination. Before each session subjects were given a pretest questionnaire to identify conditions (e.g., acute illness) that might affect their performance. The first session was a training period during which subjects were given the tests while wearing a nasal mask through which gasses were administered as described below. In previous field studies we have observed that much of the improvement in performance on those tests suitable for repeated administration occurred between the first and second session. Therefore, a single training session was given before nitrous oxide administration and control sessions were given in counterbalanced order across subjects. Both the subject and the test administrator (B. D. G.) were unaware of treatment conditions.

Subjects were tested with the NES software developed by Baker et al.[2] and a portable microcomputer. The testing apparatus was brought to the laboratory on each test day; setup time was <5 minutes. The NES is a computer-based collection of neurobehavioral tests similar to nonautomated tests of human performance used widely in clinical neuropsychology and in epidemiologic investigations of possible neurobehavioral dysfunction produced by environmental exposure to neurotoxic agents.[7] The NES software is designed to run on IBM-compatible microcomputers. The system is flexible in that different subsets of the approximately 15 available tasks can be selected, and the difficulty of the tests can be adjusted depending on the purposes of a study.

Sessions began with a computer-presented orientation sequence during which subjects learned how to respond to the COMPAQ computer (COMPAQ Computer Corp.) using the numeric keys on the keyboard (the other keys were covered by an opaque acrylic template), two buttons, and a joystick (ORA Electronics). In addition, each separate task contained instructions and practice trials. The computer continually monitored the subjects' performance, interrupting with refresher instructions or a request to call the test administrator if a subject's error rate was high. Subjects rarely made sufficient errors for this to occur, and as a group had few questions about the testing procedure. The NES tests described below were presented for each session. Administration of the tests required approximately 40 minutes for each session. At the end of the second and third sessions, subjects were asked whether they thought they had received an active agent.

NES tests. Nine NES tests were selected for this study, listed below in order of administration and according to the aspect of CNS function they are primarily intended to evaluate. These tests and the rationale underlying their use are described in more detail elsewhere.[3]

Psychomotor performance. (1) Continuous performance test: In this test of sustained visual attention, subjects press a button in response to a large letter "S" appearing randomly as part of a 5-minute series of other letters that flash briefly on the display screen at the rate of one per second. Reaction times to the target stimuli and response errors are recorded. (2) Symbol-digit substitution test: In this task the subject is presented with nine pairs of symbols and digits at the top of the screen. The subject responds by matching digits to the nine symbols, which are reordered and appear at the bottom of the screen. The time taken to match digits to the appropriate symbols and the number of incorrect responses are recorded. After a practice trial, four sets of nine symbol-digit pairs are presented in succession. To avoid learning effects, the symbol-digit pairs are different between sets. (3) Finger tapping test: In this test of simple hand speed, subjects press a button with the index finger of the left hand as many times as possible in a 10-second interval, repeat this task with the

Table I. Effects of nitrous oxide on NES performance in normal subjects (n = 12)

	Training	*Control*	*Nitrous oxide*	*Paired t test*
Continuous performance test latency (msec)	445.8 ± 31.4	448.3 ± 31.5	464.8 ± 26.2	−2.97*
Symbol-digit (sec/symbol)	1.99 ± 0.32	1.87 ± 0.33	2.00 ± 0.32	−3.42†
Finger tapping (mean no.)	50.7 ± 11.6	54.9 ± 9.3	52.1 ± 7.3	3.18†
Pattern comparison latency (sec)	2.96 ± 0.57	2.41 ± 0.67	2.41 ± 0.66	0.00
Hand-eye coordination (root mean square error)	3.68 ± 1.09	3.30 ± 0.73	3.24 ± 0.29	0.56
Associate learning (no. correct)	5.27 ± 1.10	4.91 ± 1.97	4.82 ± 1.94	0.14
Associate recall (no. correct)	7.54 ± 1.37	5.72 ± 3.00	5.45 ± 2.58	0.30
Pattern memory (no. correct)	12.6 ± 1.6	12.9 ± 1.0	12.3 ± 2.2	1.12
Digit learning (no. correct)	2.25 ± 1.22	2.58 ± 2.58	2.25 ± 2.18	0.48

Data are $\bar{X}$ ± SD.
*$P < 0.01$, †$P < 0.005$ compared with control value.

right index finger, and then press two buttons (2 cm apart) alternately with the index finger of the preferred hand. This set of tapping trials is presented twice.

Visuospatial ability. (4) Pattern comparison test: The subject is presented with three patterns (each a 10 × 10 square array), two of which are identical, and asked to select the odd pattern. This task assesses visual organization ability, a cognitive function necessary in the perception of complex visual stimuli. For each trial response times and errors are recorded. (5) Hand-eye coordination test: Using a joystick modified to move in only one dimension, subjects trace a large sine-wave pattern on the display screen. The cursor is moved horizontally at a constant rate by the computer, while the subject controls only the cursor's vertical motion with the joystick. The average vertical deviation of the cursor from the pattern is recorded as the mean absolute error and root mean square error for each trial.

Memory. (6) Paired-associate learning test: This test is designed to evaluate short-term memory. Nine associations, in which a three-letter name is paired with a one-word occupation, are presented on the monitor for 2 seconds each with 1 second between pairs. The associations are presented three times in varying order. Subjects match jobs to the appropriate name from multiple choices after each presentation of the list. The number of correct responses is scored for each trial. To avoid learning effects across sessions, different associate pairs are presented in each test session. To evaluate memory encoding and intermediate recall, a multiple-choice matching trial with the same nine name-job associations is given near the end of the session. (In this study the retention interval was approximately 30 minutes.) The number of correct responses is scored for each of the immediate and delayed recall trials. (7) Pattern memory test: A test of short-term visual memory in which subjects are briefly presented a block-like pattern (like those used in the pattern comparison test) and then required to identify which of three subsequently presented patterns is identical to the original pattern. The similarity of the three test patterns to each other is increased over the fifteen trials, so that the choice between them becomes progressively more difficult. The number of correct responses and the response time are recorded for each trial. (8) Serial digit learning: This is a test of short-term memory and attention in which subjects are presented a series of 10 digits on the display screen at a rate of one per second. Upon completion they are asked to enter that series into the computer. The series is presented until two successive trials are performed perfectly, or at most 10 times. The number of correct digits entered on each trial is recorded. The series is changed for each test session to avoid learning effects.

Mood. (9) Mood scales: Subjects were asked to describe their mood during the 40-minute test session using a five-point scale to indicate the degree to which each of 25 adjectives presented described their emotional state. Responses to five items contributed equally to five mood scales: tension, depression, anger, fatigue, and confusion.

Drug administration. During drug sessions subjects received a combination of 20% nitrous oxide and 80% oxygen from a McKesson Analor 3C nitrous oxide inhalation sedation machine through a Brown Scavenging Mask (Narco Corporation). This dose is relatively low but above the threshold for neurobehavioral dysfunction.[10,12] The drug was administered and monitored by a licensed practitioner (P. A. M.). During the training and control sessions subjects received 100% oxygen. For all sessions the mask was given a peppermint scent to disguise possible odors of the administered gasses. In all cases, subjects began the first task after breathing through the mask for 10 minutes. As previously demonstrated, this is sufficient time for blood levels of nitrous oxide to stabilize under our experimental condi-

tions.[10] After each session the mask was removed and subjects were monitored in air for at least 20 minutes until recovery was deemed complete.

Data analysis. All data were analyzed with SPSS personal computer software. Data for each behavioral measure were subjected to a t test for paired means. Because it was expected a priori that all effects of nitrous oxide would be in the direction of poorer performance, one-tailed probabilities were used. ANOVA and ANCOVA were performed to test for effects of order of drug administration and subjects' belief as to whether they received nitrous oxide.

RESULTS

Nitrous oxide significantly affected performance on three tests: the continuous performance task, symbol-digit substitution, and finger tapping (Table I). Summary measures for all three tasks showed slower performance during the nitrous oxide session than during the 100% oxygen session; that is, mean response time (of 60 trials) for the continuous performance task increased, the mean of the best two of four trials (expressed as seconds per correct symbol) for the symbol-digit substitution task increased, and the mean number of taps on the two trials when subjects alternated between buttons for the finger tapping test decreased. Performance of the other tasks (Table I) and measures of mood were not significantly affected by the dose of nitrous oxide used.

The pattern of scores across sessions was similar for most of the behavioral measures, with performance during the control (either the second or third) session better than during the training (first) session. This pattern was reversed for the associate learning and recall tests, in which poorer performance after the training session suggests a proactive interference effect (i.e., the learning of associate pairs on the first session may have interfered with subsequent acquisition of new associations). These trends illustrate the value of the training session in minimizing the potential increase in error variance resulting from a combination of the effects of repeated test presentation and drug administration.

Although our ability to assess the influence of other factors on subjects' performance was limited by the relatively small sample size, ANOVA of data for all three sessions indicated that order of treatment and subjects' belief as to whether they received an active agent had no significant influence on their performance. The subjective effects of nitrous oxide at the dose used were subtle; 25% of subjects receiving the drug believed they had received oxygen control. No subjects had excessive or idiosyncratic reactions to nitrous oxide.

DISCUSSION

Our current study was designed to assess the sensitivity of the NES in detecting neurobehavioral effects of exposure to a relatively low dose of nitrous oxide. Our observation that the continuous performance, symbol-digit substitution, and finger tapping tasks detected psychomotor impairment produced by 20% nitrous oxide are consistent with those of a number of previous studies[10,12] and indicate that the NES includes tests sensitive to the pharmacologic effects of this anesthetic. These findings may be particularly relevant to the intended use of the system in detecting neurobehavioral impairment in occupational settings. Disturbances in psychomotor functioning are frequently reported after exposure to a number of neurotoxins used in industry.[5] As discussed above, data on the effects of nitrous oxide may be especially helpful to research characterizing neurobehavioral effects of exposure to organic solvents that, like nitrous oxide, are CNS depressants and in some cases (e.g., 1,1,1-trichloroethane) were once used as inhalation anesthetics.[4]

While 20% nitrous oxide had significant effects on measures of psychomotor speed, the drug did not significantly affect performance on NES tasks designed to assess other neurobehavioral functions such as visuospatial ability (pattern recognition, hand-eye coordination), visual memory (pattern memory), verbal learning and memory (paired associates), and mood (mood scales). These findings are generally consistent with previous studies demonstrating that higher doses of nitrous oxide are required to affect such CNS functions as learning and memory consistently.[12] In addition, the sample size we used may have been too small to permit detection of subtle changes in cognitive performance suggested by the results, as there was a trend toward increased response latency in the pattern memory test. Further studies to assess the sensitivity of the various NES tests with higher nitrous oxide doses, other test agents, and larger subject groups would be useful.

Our findings are internally consistent, in that two tests sensitive to nitrous oxide exposure in this study correlated with each other when factor analysis was applied to data obtained in epidemiologic surveys of large populations. In those studies data from the continuous performance and symbol-digit tasks loaded heavily on a factor that appears related to psychomotor speed.* Although comparable field study data are not available for the finger tapping task, this test is also considered to measure psychomotor speed,[9] and a similar task has been demonstrated to be sensitive to nitrous

*Fidler AT: Unpublished observations, 1985.

oxide–induced impairment.[8] This internal consistency, together with the concordance noted above between our findings and previous work, mitigates a potential problem raised by the use of multiple comparisons in our statistical analysis (the possibility that some of the paired t tests reached statistical significance by chance). It therefore appears most likely that the effects we observed were not chance events but rather the result of the pharmacologic action of nitrous oxide.

These results provide evidence that a computer-based NES can reliably detect subtle psychomotor impairment induced by a CNS depressant (and presumably by other factors as well). The sensitivity of this system, coupled with the capability for rapid, efficient, and reliable data collection it provides, suggests that the NES holds promise for a variety of applications in epidemiologic, experimental, and clinical research. For example, as a monitor of CNS function, the NES may find application in assessing beneficial as well as toxic effects of drug therapy. While our experience with this system in the clinic does not suggest it should be used as a screening instrument, it does appear that the NES may be useful in monitoring the status of a variety of neurologic conditions (e.g., head trauma) in which severity would be expected to change over time. It is possible that the use of a common evaluation system in the laboratory, in clinical research, and in field studies could enhance progress in neurobehavioral toxicology by facilitating comparison of data obtained.

The NES software is currently available for scientific research purposes to members of the NES Users' Group at no charge. There are currently 23 users, and versions are available in English, French, Dutch, and Finnish. For information about joining the NES Users' Group, contact the last two authors.

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